DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

LUMINEX MOLECULAR DIAGNOSTICS, INC.
TINA IP
REGULATORY AFFAIRS ASSOCIATE
439 UNIVERSITY AVE.
TORONTO, ONTARIO, M5G 1Y8
CANADA

October 24, 2014

Re: K140647
Trade/Device Name: Xtag® Gastrointestinal Pathogen Panel (GPP)
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal pathogen panel multiplex nucleic acid-based assay system
Regulatory Class: II
Product Code: PCH, NSU, JJH
Dated: September 15, 2014
Received: September 16, 2014

Dear Ms. Ip:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Stephen J. Lovell -S for

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement on last page.

510(k) Number *(if known)*
K140647

Device Name
xTAG® Gastrointestinal Pathogen Panel (GPP)

Indications for Use *(Describe)*

The xTAG® Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, bacterial and parasitic nucleic acids in human stool specimens or human stool in Cary-Blair media from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP:

Viruses
- Adenovirus 40/41
- Norovirus GI/GII
- Rotavirus A

Bacteria
- *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only)
- *Clostridium difficile* (*C. difficile*) toxin A/B
- *Escherichia coli* (*E. coli*) O157
- Enterotoxigenic *E. coli* (ETEC) LT/ST
- *Salmonella*
- Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
- *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*)
- *Vibrio cholerae* (*V. cholerae*) cholera toxin gene (ctx)

Parasites
- *Cryptosporidium* (*C. parvum* and *C. hominis* only)
- *Entamoeba histolytica* (*E. histolytica*)
- *Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*)

The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG GPP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections.

The xTAG GPP test is indicated for use with the Luminex® 100/200™ and MAGPIX® instruments with xPONENT® software.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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510(k) Summary

This Summary of 510(k) information is being submitted in accordance with the requirements of 21 CFR 807.92.

510(k) Number: K140647

Submission Type: Traditional 510(k), New Device

Measurand: A panel of viruses, bacteria and parasites and toxins including: Adenovirus 40/41, Norovirus GI/GII, Rotavirus A, *Campylobacter* (*C. jejuni, C. coli* and *C. lari* only), *Clostridium difficile* toxin A/B, *Escherichia coli* (*E. coli*) O157, Enterotoxigenic *E. coli* (ETEC) LT/ST, *Salmonella*, Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2, *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*), *Vibrio cholerae (V. cholerae)* cholera toxin gene (ctx), *Cryptosporidium* (*C. parvum* and *C. hominis* only), *Entamoeba histolytica* (*E. histolytica*), *Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*) and the internal control (bacteriophage MS2).

Type of Test: Qualitative nucleic acid multiplex test

Applicant: Luminex Molecular Diagnostics, Inc., Toronto, Ontario, Canada

Proprietary and Established Names: xTAG® Gastrointestinal Pathogen Panel (GPP)

Regulatory Information:

Product Code	*Classification*	*Regulation Section*	*Review Panel*
PCH	II	21CFR866.3990 Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System	Microbiology (83)
NSU	II	21CFR862.2570 Multiplex Instrument System	Microbiology (83)

Device Components

Product	**Description**
xTAG® GPP Kit	Unchanged from k121894
xTAG® GPP TDAS (Software CD)	Revised CD, containing data acquisition protocol and data analysis software (updated to include Adenovirus 40/41, *V. cholerae* and *E. histolytica*)
Luminex® MAGPIX® instrument	Unchanged from k121894
xPONENT® Software	xPONENT® Software unchanged from k121894

Intended Use:

The xTAG® Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, bacterial, and parasitic nucleic acids in human stool specimens or human stool in Cary-Blair media from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP:

Viruses
- Adenovirus 40/41
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- Rotavirus A

Bacteria
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- *Clostridium difficile* (*C. difficile*) toxin A/B
- *Escherichia coli* (*E. coli*) O157
- Enterotoxigenic *Escherichia coli* (ETEC) LT/ST
- *Salmonella*
- Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2
- *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*)
- *Vibrio cholerae* (*V. cholerae*) cholera toxin gene (ctx)

Parasites
- *Cryptosporidium* (*C. parvum* and *C. hominis* only)
- *Entamoeba histolytica* (*E. histolytica*)
- *Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*)

The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG GPP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections.

The xTAG GPP test is indicated for use with the Luminex® 100/200™ and MAGPIX® instruments with xPONENT® software.

Indication(s) for use: Same as intended use.

Special instrument requirements: Luminex MAGPIX instrument with xPONENT software.

Device Description:
xTAG GPP incorporates a multiplex reverse-transcription polymerase chain reaction (RT-PCR) with Luminex's proprietary universal sorting system (the xTAG Universal Array) on the Luminex platform. The xTAG Universal Array sorts nucleic acids onto discreet Luminex bead populations by virtue of highly specific "tag/anti-tag" hybridization reactions. The tags and anti-tags comprising the xTAG Universal Array are 24-mer oligonucleotide sequences not found in nature. The assay has been designed to simultaneously detect microbial targets and an internal control (bacteriophage MS2 added to each sample prior to extraction).

For each sample, 10 µL of extracted nucleic acid is amplified in a single multiplex RT-PCR reaction. Amplimers ranging from 58 to 202 bp (not including the 24-mer tag) are generated in this reaction. A five µL aliquot of the RT-PCR product is then subjected to a hybridization/detection reaction that also includes bead populations coupled to 24-mer anti-tags. Each bead population is coupled to a unique anti-tag which is the exact complement of a 24-mer tag incorporated into a given amplimer. Thus, each Luminex bead population uniquely identifies a microbial target or assay control through a specific tag/anti-tag hybridization reaction. Signal is generated via a Streptavidin, R-Phycoerythrin conjugate.

The Luminex instrument sorts the products of these hybridization reactions and generates a signal in the form of a median fluorescence intensity (MFI) value for each bead population. The MFI values are generated by the xPONENT software provided with the instrument using the GPP protocol parameters, and are analyzed by the xTAG Data Analysis Software (TDAS GPP (US)). TDAS GPP (US) applies algorithms to MFI values in order to generate a qualitative result for each microbial target selected for reporting to establish the presence or absence of bacterial, viral or parasitic targets and/or controls in each sample. The data analysis software also generates a qualitative result and compiles a report for patient samples and external controls assayed in a given run. Before data are analyzed, a user has the option to select a subset of the targets from the intended use of the xTAG GPP (for each sample).

Substantial Equivalence Information:

Table 1: Similarities between New Device and Predicate

Item	New Device (k140647) xTAG GPP	Predicate (k121894) xTAG GPP
Manufacturer (Same)	Luminex Molecular Diagnostics	Luminex Molecular Diagnostics
Extraction Method (Same)	bioMérieux NucliSENS® easyMAG®	bioMérieux NucliSENS easyMAG
Test Principle and Amplification Method (Same)	Multiplex end point RT-PCR	Multiplex end point RT-PCR
Kit Reagents (Same)	xTAG GPP Primer Mix, xTAG OneStep Enzyme Mix, xTAG® OneStep Buffer, xTAG RNase-Free Water, xTAG BSA, xTAG MS2, xTAG® GPP Bead Mix, xTAG Reporter Buffer, xTAG 0.22 SAPE	xTAG GPP Primer Mix, xTAG OneStep Enzyme Mix, xTAG OneStep Buffer, xTAG RNase-Free Water, xTAG BSA, xTAG MS2, xTAG GPP Bead Mix, xTAG Reporter Buffer, xTAG 0.22 SAPE
Test Format (Same)	Multiplex MAGPLEX bead-based universal array	Multiplex MAGPLEX bead-based universal array
Detection Method (Same)	Fluorescence based	Fluorescence based
Quality Control (Same)	Internal Control (MS2), rotating analyte controls and negative control (RNAse-free water)	Internal Control (MS2), rotating analyte controls and negative control (RNAse-free water)
Results (Same)	Qualitative	Qualitative
Instrument Software System	Luminex MAGPIX with xPONENT Software	Luminex MAGPIX with xPONENT Software

Table 2: Differences between New Device and Predicate

Item	New Device (k140647) xTAG GPP	Predicate (k121894) xTAG GPP
Specimen Types	Human stool specimens and human stool in Cary-Blair media	Human stool specimens
Software	Updated assay protocol to acquire and show data for additional 3 analytes: Adenovirus 40/41, *Entamoeba histolytica* (*E. histolytica*), and *Vibrio cholerae* (*V. cholerae*).	Assay protocol file excludes analytes Adenovirus 40/41, *Entamoeba histolytica* (*E. histolytica*), and *Vibrio cholerae* (*V. cholerae*)
Intended Use	See above. Addition of sample type human stool in Cary-Blair media and addition of analytes Adenovirus 40/41, Entamoeba histolytica (*E. histolytica*), and *Vibrio cholerae* (*V. cholerae*) cholera toxin gene (*ctx*). Specified software used with Luminex 100/200 (k140377) and MAGPIX instruments. Organized analytes listed under sub-heading of viruses, bacteria and parasites.	The xTAG Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG® GPP: • *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only) • *Clostridium difficile* (*C. difficile*) toxin A/B • *Cryptosporidium* (*C. parvum* and *C. hominis* only) • *Escherichia coli* (*E. coli*) O157 • Enterotoxigenic *Escherichia coli* (ETEC) LT/ST • *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*)

Item	New Device (k140647) xTAG GPP	Predicate (k121894) xTAG GPP
		• Norovirus GI/GII • Rotavirus A • *Salmonella* • Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2 • *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*) The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks. **xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.** The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. **xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections.** The xTAG GPP is indicated for use with the Luminex MAGPIX instrument.
Targets Reported	**Adenovirus 40/41**, *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only), *Clostridium difficile* (*C. difficile*) toxin A/B, *Cryptosporidium* (*C. parvum* and *C. hominis* only), *Escherichia coli* (*E. coli*) O157, Enterotoxigenic *Escherichia coli* (ETEC) LT/ST, ***Entamoeba histolytica* (*E. histolytica*)**, *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*), Norovirus GI/GII, Rotavirus A, *Salmonella,* Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2, *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*), ***Vibrio cholerae* (*V. cholerae*)** cholera toxin gene (ctx)	*Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only), *Clostridium difficile* (*C. difficile*) toxin A/B, *Cryptosporidium* (*C. parvum* and *C. hominis* only), *Escherichia coli* (*E. coli*) O157, Enterotoxigenic *Escherichia coli* (ETEC) LT/ST, *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*), Norovirus GI/GII, Rotavirus A, *Salmonella,* Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2, *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*)

Standards/Guidance Documents referenced (if applicable):

Table 3: Guidance Documents

	Title	Date
1	Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of *Clostridium difficile*	Nov. 29, 2010
2	Class II Special Controls Guidance Document: Norovirus Serological Reagents	Mar. 9, 2012
3	Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems - Guidance for Industry and FDA Staff	Mar. 10, 2005
4	Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices	May 11, 2005
5	Guidance document for Format for Traditional and Abbreviated 510(k)s	Aug. 12, 2005
6	Guidance on the CDRH Premarket Notification Review Program, 510(k) Memorandum #K86-3	June 30, 1986
7	The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications - Final Guidance	Mar. 20, 1998
8	The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]	Dec. 27, 2011
9	Guidance for Industry and Food and Drug Administration Staff - eCopy Program for Medical Device Submissions	Oct. 10, 2013
10	Guidance for Industry and Food and Drug Administration Staff - FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Goals	Oct. 15, 2012

Table 4: Standards

	Standard No.	Recognition Number (FDA)	Standards Title	Date
1	EP05-A2	7-110	Evaluation of Precision Performance of Quantitative measurement Methods (2nd ed.)	10/31/2005
2	EP07-A2	7-127	Interference Testing in Clinical Chemistry (2nd edition)	05/21/2007
3	EP12-A2	7-152	User Protocol for Evaluation f Qualitative Test Performance (2nd edition)	09/09/2008
4	EP14-A2	7-143	Evaluation of Matrix Effects (2nd edition)	03/16/2012
5	EP15-A2	7-153	User Verification of Performance for Precision and Trueness (2nd edition)	09/09/2008
6	EP17-A	7-194	Protocol for Determination of Limits of Detection and Limits of Quantitation (NOTE: Original studies included this standard)	03/28/2009
7	EP17-A2	7-233	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures	01/15/2013
8	ISO 14971	5-40	Application of Risk Management to Medical Devices	08/20/2012
9	MM03-A2	7-132	Molecular Diagnostic Methods for Infectious Diseases (2nd edition)	09/09/2008

	Standard No.	Recognition Number (FDA)	Standards Title	Date
10	MM13-A	7-191	Collection, Transport, Preparation and Storage of Specimens	03/18/2009

Analytical Performance:

The reagents tested in submission k121894 remain the same as the reagents used in testing performed towards this submission. Therefore, the study reports and results presented in the submission summary in k121894 for Analytical Reactivity, Carry-Over Contamination, Limit of Detection, Repeatability, Analytical Specificity (including Interference), Evaluation of Fresh vs. Frozen Stool, and Reproducibility / Precision are all still applicable to the new device. Results presented below for each of these studies are additive to results previously presented in k121894 and include results for Adenovirus 40/41, *Entamoeba histolytica* (*E. histolytica*), and *Vibrio cholerae* (*V. cholerae*) cholera toxin gene (ctx). This section of the summary includes updated results for these three analytes for the following studies:

1. Analytical Reactivity
2. Carry-over Contamination
3. Limit of Detection
4. Repeatability
5. Analytical Specificity and Interference
6. Evaluation of Fresh vs. Frozen Stool
7. Reproducibility / Precision

Additionally, a study demonstrating results of testing analytes in stool as compared to stool in Cary-Blair media is presented, at the limit of detection for each analyte to demonstrate that either sample type can be used with xTAG GPP.

Finally, a summary of negative control failures and sample re-run rates for analytical performance studies is provided.

Analytical Reactivity

Analytical reactivity was assessed through empirical testing of a wide range of clinically relevant GI pathogen strains, genotypes, serotypes and isolates representing temporal and geographical diversity for each analyte. Through testing of unique samples covering the additional intended use pathogens, reactivity was established at concentrations 2 to 3 times the limit of detection.

Adenovirus - The Limit of Detection (LoD) using Adenovirus 40, Zeptometrix 0810084CF (Dugan) and Adenovirus 41, Zeptometrix 0810085CF (Tak) were found to be 1.45E+01 $TCID_{50}$/mL (or 4.89E+06 Copies/mL) and 7.69E+00 $TCID_{50}$/mL (or 1.48E+07 Copies/mL), respectively (see LoD section below). The following two samples were tested at the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA). Note: these samples were different isolates of the strains used in the LoD study (See LoD section below). The amount of the viral target DNA for GP-093 and GP-094 was measured by real-time PCR and the Ct values generated were used to calculate the DNA copy number. The lowest reactivity titers for GP-093 and GP-094, were found to be at 3x and 1x multiple of LoD level, respectively.

Table 5: Adenovirus Reactivity List

Run Batch ID	Target	Source ID	Strain or Serotype	Reactivity Titer (Copies/mL)	Results Summary
Analytical reactivity_II	Adenovirus 40	CDC – GP-093	Dugan $pCMK_2Gr_{10,}$ 9/23/91	1.49E+07	POS
Analytical reactivity_II	Adenovirus 41	CDC – GP-094	Tak $HeLa_2Gr_{10,}$ 9/23/91	1.43E+07	POS

Furthermore, in sequencing analysis of clinical specimens tested as part of the multi-site clinical study of xTAG GPP, 9 Adenovirus 40 and 28 Adenovirus 41 positive samples were detected by the assay and sequencing.

Table 6: Adenovirus Clinical Specimen Positive by the xTAG GPP

Target	Clinical Sample ID
Adenovirus 40	GPP03-092B, GPP03-099B, GPP03-101B, GPP03-102B, GPP03-103B, GPP03-106B, GPP03-109B, GPP03-300B, GPP03-240B
Adenovirus 41	GPP03-001B, GPP03-003B, GPP03-007B, GPP03-013B, GPP03-014B, GPP03-019B, GPP03-020B, GPP03-022B, GPP03-025B, GPP03-026B, GPP03-028B, GPP03-029B, GPP03-033B, GPP03-035B, GPP03-036B, GPP03-037B, GPP03-038B, GPP03-039B, GPP03-048B, GPP03-055B, GPP03-060B, GPP03-095B, GPP03-229B, GPP03-313B, GPP04-159, GPP04-174, GPP02-129, GPP02-192

***Entamoeba histolytica* -** The LoD for *Entamoeba histolytica*, ATCC 30890 was found to be 2.88E+01 Cells/mL, equivalent to 4.30E+02 Copies/mL (see LoD section below). For *E.histolytica*, ATCC 50007, 50481, 50738 and 50454, the titer information expressed in Cells/mL could not be obtained. To standardize the quantification units for all *E.histolytica* strains, in this Analytical Reactivity study the amount of target DNA was measured by real-time PCR and the Ct values generated were used to calculate the DNA copy numbers. The reactivity titers for most of the strains were in the range of 0.4x to 6.7x multiple of LoD level for *E.histolytica*. The reactivity titer

for ATCC 50738 (Rahman) was found to be 0.2x multiple of LoD level. Table 7: *Entamoeba histolytica* Reactivity List

Run Batch ID	Target	Source	Strain or Serotype	Reactivity Titer (Cells or Copies/mL)	Results Summary
20120216_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30015	(HK-9, colonic biopsy from adult human male with amebic dysentery, Korea); frozen	2.86E+00 Cells/mL, or 1.82E+02 Copies/mL	POS
20120216_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30190	(HB-301:NIH, feces from adult human male with amebic dysentery, Burma, 1960); test tube	1.07E+03 Copies/mL	POS
20120216_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30457	(HU-21:AMC, colonic biopsy from male child with amebic dysentery, Little Rock, AR, 1970); test tube	1.68E+03 Copies/mL	POS
20120216_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30458	(200:NIH); frozen	1.83E+02 Cells/mL, or 2.42E+03 Copies/mL	POS
20120216_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30459	(HM-1:IMSS [ABRM]; feces from adult human male, asymptomatic cyst passer, England, 1972); test tube	1.83E+02 Cells/mL, or 1.10E+03 Copies/mL	POS
20120314_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 30889	(H-458:CDC [ATCC30217], feces from human adult female with amebic dysentery, Asia (?), (patient in U.S. for treatment), 1971); test tube	8.78E+02 Copies/mL	POS
20120411_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 30923	(HU-2:MUSC)	1.61E+03 Copies/mL	POS
20120207_JF_GPP_Reactivity_MP	*Entamoeba histolytica*	ATCC 30925	(HU-1:CDC, feces of female child, asymptomatic, sero-negative cyst passer, Cherokee, NC, 1978)	1.89E+02 Copies/mL	POS
20120411_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 50007	DKB	2.88E+03 Copies/mL	POS
20120411_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 50481	SD157	1.36E+03 Copies/mL	POS
20120411_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 50738	Rahman	8.90E+01 Copies/mL	POS
20120411_JF_GPP_React_MP	*Entamoeba histolytica*	ATCC 50454	HB-301:NIH	1.08E+03 Copies/mL	POS

Vibrio cholerae **-** The LoD using *Vibrio cholerae Pacini* ATCC 14101 (serovar O:1) was found to be 2.34E+06 CFU/mL. For this Analytical Reactivity study 3xLoD=7.02E+06 CFU/mL, and this was used for initial reactivity testing. In addition to toxinogenic strains, (i.e. O1 and O139), the xTAG GPP assay also detects any non:O1 *Vibrio cholerae* strains that do express cholera toxin gene, ctx (xTAG GPP *Vibrio cholerae* primers target gene), but not the non:O1 strains that may cause clinical symptoms such as diarrhea by expressing a different virulence factor, which is likely the case for sample ATCC 14374 and other non:O1 strains in this table. Both non-O1 ATCC 25872 and non-O1 ATCC 25873 strains, were tested in sequencing assays and confirmed to contain the ctx gene with well conserved xTAG GPP *Vibrio cholerae* primer binding regions.

Table 8: *Vibrio cholerae* Reactivity List

Run Batch ID	Target	Source	Strain or Serotype	Reactivity Titer (CFU/mL)	Results Summary
20120827-JX-V cholera-AR-MP	*Vibrio cholerae Pacini*	NCTC 30	Non-O:1, ATCC 4735;MARTIN 1	6.00E+08	NEG
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 4714	Non-O:1, Isolated from pilgrims in El Tor quarantine camp, El Tor 34-D 19	6.00E+08	NEG
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 7260	O:1, EGYPT 117	7.02E+06	POS
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 11500	Non-O:1, VL 7050	6.00E+08	NEG
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 11507	Non-O:1, VL 1941	6.00E+08	NEG
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 11510	O:1, VL 01211	7.02E+06	POS
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 12945	O:139 (Non-O:1 (NAG) – reference strain for O:139 serovar	7.02E+06	POS
20120827-JX-V cholera-AR-MP	*Vibrio cholerae*	NCTC 12946	O:139 (Non-O:1 (NAG))	7.02E+06	POS
20120406-JX-AnaReact-Vibrio2-MP	*Vibrio cholerae Pacini*	ATCC 14033	O:1, El Tor DO 1930;CN 5774;R. Hugh 1092, Serotype Inaba, Non-toxinogenic	1.50E+08	NEG
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae asiaticae (Trevisan) Pfeiffer*	ATCC 14035	O:1, Serotype Ogawa [7787]	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 14101	O:1, Serotype Ogawa, clinical specimen – human ([185754] cholera epidemic circa 1960, Calcutta) Calcutta India	7.02E+06	POS
20120406-JX-AnaReact-Vibrio2-MP	*Vibrio cholerae Pacini*	ATCC 14374	Non-O:1 (NAG), 5035; R. Hugh 1513	1.50E+08	NEG
20120921-MB-VibrioAnalytical-MP	*Vibrio cholerae Pacini*	ATCC 14730	Non-O:1 (Serovar O:2), biovar El Tor, Subgroup III of Gardner and Venkatraman, NCTC 4711, NANKING	6.00E+08	NEG

Run Batch ID	Target	Source	Strain or Serotype	Reactivity Titer (CFU/mL)	Results Summary
			32/123		
20120921-MB-VibrioAnalytical-MP	*Vibrio cholerae Pacini*	ATCC 14731	Non-O:1, (Serovar O:3), biovar El Tor, Subgroup V of Gardner and Venkatraman, NCTC 4715, El Tor 34-D 23;CN 3426	6.00E+08	NEG
20120921-MB-VibrioAnalytical-MP	*Vibrio cholerae Pacini*	ATCC 14732	Non-O:1 (Serovar O:4), biovar El Tor, Subgroup VI of Gardner and Venkatraman, NCTC 4716, KASAULI 73	6.00E+08	NEG
20120921-MB-VibrioAnalytical-MP	*Vibrio cholerae Pacini*	ATCC 14733	Non-O:1 (Serovar O:7), biovar El Tor, Subgroup II of Gardner and Venkatraman, NCTC 8042, NANKING 32/124	6.00E+08	NEG
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 25870	O:1, Serotype Inaba	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 25872	Non-O:1 (NAG), Isolated from a patient with clinical cholera	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 25873	Non-O:1 (NAG), Isolated from a patient with clinical cholera	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 51394	O:139 (Non-O:1 [NAG]), Cholera patient, Madras, India	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae Pacini*	ATCC 51395	O:139 (non O:1 [NAG]), clinical specimen – human (cholera patient, Madras, India)	7.02E+06	POS
20120404-JX-AnaReact-Vibrio-MP	*Vibrio cholerae*	ATCC BAA-2163	O:1, Isolated from a patient in Artibonite Department, Haiti, October 2010, Serotype Ogawa, Biogroup El Tor cholera toxin positive CDC Isolate 2010 EL-1786	7.02E+06	POS

Table 9 summarizes the samples reactive with xTAG GPP. Note that in addition to toxinogenic strains, i.e. O1 and O139, xTAG GPP assay detects any non:O1 *Vibrio cholerae* strains that do express cholera toxin gene (xTAG GPP *Vibrio cholerae* primers target gene), but not the non:O1 strains that may cause clinical symptoms such as diarrhea by expressing a different virulence

factor, which is likely the case for ATCC 14374 and other non:O1 strains tested in this study. Both non-O1 ATCC 25872 and non-O1 ATCC 25873 strains, were tested in sequencing assays and confirmed to contain the ctx gene with well conserved xTAG GPP *Vibrio cholerae* primer binding regions. Ten *Vibrio cholerae* strains that did not react with xTAG GPP assay are listed in Tables 8 and 10.

Table 9: Reactivity of Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae*

Pathogen	ATCC / Other Reference	Pathogen	ATCC / Other Reference
Adenovirus 40	CDC – GP-093	Adenovirus 41	GPP03-095B
Adenovirus 40	GPP03-092B	Adenovirus 41	GPP03-229B
Adenovirus 40	GPP03-099B	Adenovirus 41	GPP03-313B
Adenovirus 40	GPP03-101B	Adenovirus 41	GPP04-159
Adenovirus 40	GPP03-102B	Adenovirus 41	GPP04-174
Adenovirus 40	GPP03-103B	Adenovirus 41	GPP02-129
Adenovirus 40	GPP03-106B	Adenovirus 41	GPP02-192
Adenovirus 40	GPP03-109B	*Entamoeba histolytica*	ATCC 30015
Adenovirus 40	GPP03-240B	*Entamoeba histolytica*	ATCC 30190
Adenovirus 40	GPP03-300B	*Entamoeba histolytica*	ATCC 30457
Adenovirus 41	CDC – GP-094	*Entamoeba histolytica*	ATCC 30458
Adenovirus 41	GPP03-001B	*Entamoeba histolytica*	ATCC 30459
Adenovirus 41	GPP03-003B	*Entamoeba histolytica*	ATCC 30889
Adenovirus 41	GPP03-007B	*Entamoeba histolytica*	ATCC 30923
Adenovirus 41	GPP03-013B	*Entamoeba histolytica*	ATCC 30925
Adenovirus 41	GPP03-014B	*Entamoeba histolytica*	ATCC 50007
Adenovirus 41	GPP03-019B	*Entamoeba histolytica*	ATCC 50481
Adenovirus 41	GPP03-020B	*Entamoeba histolytica*	ATCC 50738
Adenovirus 41	GPP03-022B	*Entamoeba histolytica*	ATCC 50454
Adenovirus 41	GPP03-025B	*Vibrio cholerae,* serovar O:1	NCTC 7260
Adenovirus 41	GPP03-026B	*Vibrio cholerae,* serovar O:1	NCTC 11510
Adenovirus 41	GPP03-028B	*Vibrio cholerae,* serovar O:139 (Non-O:1 (NAG)) – reference strain for O:139 serovar	NCTC 12945
Adenovirus 41	GPP03-029B	*Vibrio cholerae,* serovar O:139 (Non-O:1 (NAG))	NCTC 12946
Adenovirus 41	GPP03-033B	*Vibrio cholerae asiaticae (Trevisan) Pfeiffer,* serovar O:1, serotype Ogawa	ATCC 14035
Adenovirus 41	GPP03-035B	*Vibrio cholerae Pacini,* serovar O:1, Serotype Ogawa	ATCC 14101
Adenovirus 41	GPP03-036B	*Vibrio cholerae Pacini,* serovar O:1, Serotype Inaba	ATCC 25870
Adenovirus 41	GPP03-037B	*Vibrio cholerae Pacini,* serovar Non-O:1 (NAG)	ATCC 25872
Adenovirus 41	GPP03-038B	*Vibrio cholerae Pacini,* serovar Non-O:1 (NAG)	ATCC 25873
Adenovirus 41	GPP03-039B	*Vibrio cholerae Pacini,* serovar O:139 (Non-O:1 [NAG])	ATCC 51394
Adenovirus 41	GPP03-048B	*Vibrio cholerae Pacini,* serovar O:139 (Non-O:1 [NAG])	ATCC 51395
Adenovirus 41	GPP03-055B	*Vibrio cholera,* serovar O:1, serotype Ogawa, biovar El Tor, cholera toxin positive	ATCC BAA-2163
Adenovirus 41	GPP03-060B		

Table 10: *Vibrio cholerae* Strains that did not React with xTAG GPP

Pathogen	ATCC / Other	Pathogen	ATCC / Other

	Reference		Reference
Vibrio cholerae Pacini, Serovar Non-O:1 (NAG)	NCTC 30	*Vibrio cholerae Pacini,* Serovar Non-O:1 (NAG)	ATCC 14374
Vibrio cholerae, Serovar Non-O:1	NCTC 4714	*Vibrio cholerae Pacini,* Serovar O:2, biovar El Tor, Subgroup III of Gardner and Venkatraman	ATCC 14730
Vibrio cholerae, Serovar Non-O:1	NCTC 11500	*Vibrio cholerae Pacini,* Serovar O:3, biovar ElTor, Subgroup V of Gardner and Venkatraman	ATCC 14731
Vibrio cholerae, Serovar Non-O:1	NCTC 11507	*Vibrio cholerae Pacini,* Serovar O:4, biovar El Tor; Subgroup VI of Gardner and Venkatraman	ATCC 14732
Vibrio cholerae Pacini, Serovar O1, biotype El Tor, serotype Inaba, non-toxinogenic	ATCC 14033	*Vibrio cholerae Pacini,* Serovar O:7, biovar El Tor; Subgroup II of Gardner and Venkatraman	ATCC 14733

Carry-over Contamination

The likelihood of carry-over contamination events was initially assessed and presented in k121894 by testing 2 representative pathogens (a bacteria and a parasite): *C. difficile*, and *Giardia* respectively. In this study, a representative virus (Adenovirus 40) was tested. This analyte was examined in the form of simulated samples prepared at concentrations just below the assay cut-off (High Negative, HN) and well above the assay cut-off (High Positive, HP). The target was examined in a set of 6 independent extractions. Each extraction was assayed in duplicate arranged in a checkerboard manner on a 96-well plate using xTAG GPP.
As with the results in k121894 for the representative bacteria (*C. difficile*) and parasite (*Giardia*), results with the virus (Adenovirus 40) showed that all 144 high negative samples remained negative when run on the Luminex 100/200 instrument for all three targets (100% HN). In addition, results for Adenovirus 40 showed that all 144 high positive samples remained positive when run on the Luminex 100/200 instrument (100% HP), as with the targets previously tested. Therefore a lack of carryover contamination has been demonstrated.

Limit of Detection

As in the original study results presented for k121894, the LoD was assessed by analyzing serial dilutions of simulated samples made from high-titer stocks of commercial strains or high-titer clinical specimens (when commercial strains were not available). All simulated specimens were prepared in negative clinical matrix (stool). The data from serial dilutions were confirmed in at least 20 replicates of the selected dilution for each analyte target. Results of testing for the three additional analytes were as follows:

Table 11: Summary of Limit of Detection (LoD) for Additional Analytes

Analyte	Strain ID	Titer (corresponding to the estimated LoD)	Average MFI Value	%CV
Adenovirus 40/41	Adenovirus 40, 0810084CF (Dugan)	$1.45x10^1$ $TCID_{50}$/mL	686	34.26%
	Adenovirus 41, 0810085CF (Tak)	7.69 $TCID_{50}$/mL	389	20.27%
Entamoeba histolytica	*Entamoeba histolytica*, 30890	$2.88x10^1$ cells/mL	1103	17.77%
Vibrio cholerae	*Vibrio cholerae*, 14101 (Serovar O:1)	$2.34x10^6$ CFU/mL	309	23.94%

Repeatability

As in the original study results presented for k121894, repeatability was assessed for each target by testing 20 replicates of each of two different analyte concentrations: a very low positive sample (at the LoD) and a moderate positive dilution level (5x-10x above the cut-off MFI). All replicates for each dilution level were examined starting from sample extraction with the bioMérieux NucliSENS easyMAG system followed by xTAG GPP in a single run. For each set of 20 replicates, the same operator performed the testing on the same instrument system, using the same lot of extraction kit and xTAG GPP reagents. Results of testing were as follows:

Table 12: Assay Repeatability Assessed by Confirmation of Calls

Analyte	Dilution Level	Concentration	xTAG GPP Calls	Mean MFI Value	%CV
Adenovirus 40/41	Moderate Positive	$5.80x10^1$ $TCID_{50}$/mL	20 of 20 POS	1562	8.60%
	Low Positive/LoD	$1.45x10^1$ $TCID_{50}$/mL	20 of 20 POS	686	33.39%
Entamoeba histolytica	Moderate Positive	$5.76x10^1$ cells/mL	20 of 20 POS	886	8.73%
	Low Positive/LoD	$2.88x10^1$ cells/mL	20 of 20 POS	1103	17.32%
Vibrio cholerae	Moderate Positive	$4.68x10^6$ CFU/mL	20 of 20 POS	504	15.48%
	Low Positive/LoD	$2.34x10^6$ CFU/mL	20 of 20 POS	309	23.33%

The correct qualitative result was obtained for 20 of 20 replicates at the low positive level and for 20 of 20 replicates at the moderate positive level for each analyte tested at these concentrations.

Analytical Specificity and Potential Interfering Agents

Analytical specificity was assessed with respect to the following:

1. *Propensity for cross-reactivity leading to false positive results*: Potential cross reactivity with pathogens (viruses, bacteria and parasites) associated with gastrointestinal (GI) infections that are not probed by the assay. Potential cross reactivity was also assessed for commensal flora and non-microbial agents. Organisms were tested at high positive titers.
2. *Propensity for interference leading to false negative results*: Potential interference by pathogens (viruses, bacteria and parasites) associated with gastrointestinal (GI) infections that are not probed by the assay. Potential interference by commensal flora was also assessed. Panel analytes were tested at low positive concentrations in the presence of highly concentrated non-panel organisms.
3. *Propensity for competitive interference leading to false negative results*: Potential interference by GI pathogens that are detected by the assay was evaluated by testing one microbial target prepared at a concentration near the assay cut-off (LP) in the presence of a second microbial target prepared at a very high concentration (HP), and vice-versa. The combinations of analytes tested were selected based on the frequency of co-infections reported in the literature.

Results for the 3 categories of testing outlined above were detailed in the decision summary presented for submission k121894.

The following additions relevant to results for the additional 3 analytes are included here:

Two strains of *Entamoeba dispar*, ATCC PRA-353 and PRA-368, were tested as commensal flora for potential cross-reactivity with xTAG GPP Assay (Table 13), in addition to *Entamoeba dispar* PRA-260 included in k121894. One of the three *E.dispar* strains, ATCC PRA-353, tested at 3.0E+05 cells/mL (or over 10^4 times LoD for *E. histolytica*) cross-reacted with *E.histolytica*. Testing at 4-fold lower titer (equivalent to 2.6E+03 multiples of *E. histolytica* LoD) did not produce a false-positive call. *E. histolytica* xTAG GPP kit primers were analyzed in silico for cross-reactivity with *E.dispar*. Two *E. dispar* sequences were available in Genbank, Z49256 (unknown strain) and AB282661 (strain SAW1734Rc1AR). In addition, three ATCC strains, PRA-260, PRA-353 and PRA-368, were sequenced at Luminex with primers flanking the xTAG GPP kit *E. histolytica* primer binding region. All five *E. dispar* sequences were identical in the *E. histolytica* GPP kit amplicon region. The forward primer was a perfect match to the *E. dispar* sequences, whereas the reverse primer had multiple mismatches, most notably, a 2-nt contiguous mismatch on the 3' end. These mismatches in the reverse primer would cause a significant decrease in amplification efficiency, and, therefore, result in a negligible risk of obtaining a false-positive xTAG GPP result for *E. histolytica*.
As the xTAG GPP testing demonstrated, a false-positive call is only possible when *E. dispar* is present at a very high concentration, 3.0E+05 cells/mL (or over 10^4 times LoD for *E. histolytica*) or higher. Testing at 4-fold lower titer (equivalent to 2.4E+03 to 2.6E+03 multiples of *E. histolytica* LoD) does not produce a false-positive call.

Table 13: Cross-reactivity of xTAG GPP Assay with non-Panel Organisms (Commensal Flora)

Commensal Flora	ATCC/Other Reference	Titer Tested	Cross-Reactive Yes (Y) / No (N)
Entamoeba dispar	ATCC PRA-260	6.80E+06 copies/mL	N
Entamoeba dispar	ATCC PRA-353	3.00E+05 cells/mL	Y
Entamoeba dispar	ATCC PRA-353	7.50E+04 cells/mL	N
Entamoeba dispar	ATCC PRA-368	7.00E+04 cells/mL	N

Astrovirus was used as a representative interfering pathogen associated with gastrointestinal (GI) infections that are *not* probed by the assay (See Table 14). The xTAG GPP analyte, in this case Adenovirus 40/41, was also run without a second analyte present. No interference was seen.

Non-panel interference with common commensal bacteria, yeast and parasites was evaluated for each target in the xTAG GPP assay. Organisms tested are presented in Table 15 below. Low positive samples of each analyte target in the assay were tested in the presence of a high positive sample of the potential interfering microorganism. All non-panel bacteria and yeast were tested at a concentration of 6E+08 cfu/mL except for *Blastocystis hominis* (ATCC 50587 - concentration ≥ 1E+06 cells/mL and ATCC 50608 - concentration 2.00E+07 cells/mL). No interference was found with the xTAG GPP analytes Adenovirus, *Entamoeba histolytica* and *Vibrio cholerae*.

Table 14: Interference with Non-Panel Gastrointestinal Pathogens

xTAG GPP Analyte (concentration)	Source	Potentially Interfering Organism (concentration)	Source	Interference Yes (Y) /No (N)
Adenovirus serotypes 40 (LP) (1.49E+07 copies/mL)	CDC	None		N
		Astrovirus (HP) (6.00E+10 copies/mL)	CDC	N
Adenovirus serotypes 41 (LP) (1.43 E+07 copies/mL)	CDC	None		N
		Astrovirus (HP) (6.00 E+10 copies/mL)	CDC	N

Table 15: Common Commensal Bacteria, Yeast and Parasites Tested for Interference

Pathogen
Bacteroides thetaiotaomicron (ATCC 29148)
Citrobacter koseri (ATCC 27028)
Clostridium sporogenes (ATCC 3584)
E. coli strain ECOR2 (ATCC 35321)
Enterobacter cloacae (ATCC 13047)
Klebsiella pneumoniae subsp. *pneumoniae* (ATCC 13883)
Pseudomonas putida (ATCC 47054)
Proteus penneri (ATCC 35198)
Candida albicans (ATCC 10231)
Blastocystis hominis (ATCC 50587 or 50608)

Potential interference with GI pathogens that are a part of the assay (competitive interference) was evaluated with one target prepared at a concentration near the assay cut-off (LP) and the other target prepared at a very high concentration (HP) and vice versa. In each case, xTAG GPP Analyte 1 was also run without a second analyte present. Results (interference in making the appropriate calls) are shown in Table 16. There was no competitive interference observed between pathogens probed by xTAG GPP when testing was carried out with the mixed analyte samples described below.

Table 16: Competitive Interference with Panel Pathogens

xTAG GPP Analyte #1	xTAG GPP Analyte #2
Adenovirus serotype 40 (HP) (3.80E+06 $TCID_{50}$/mL)	No Analyte #2
	Norovirus (LP) (160x dilution of stock)
	Salmonella enterica subsp. *enterica* serovar Typhimurium (LP) (8.78E+04 cfu/mL)
	Campylobacter jejuni (LP) (2.93E+05 cfu/mL)
Adenovirus serotype 40 (LP) (5.25E+01 $TCID_{50}$/mL)	No Analyte #2
	Norovirus (HP)
	Salmonella enterica subsp. *enterica* serovar Typhimurium (HP) (6.00E+08 cfu/mL)
	Campylobacter jejuni (HP) (6.00E+08 cfu/mL)

The pathogens listed in Table 17 were not attainable. However, an *in silico* analysis was performed to assess the potential for non-specific cross-reactivity of these microbial pathogens with the primers used in xTAG GPP (BLAST results located in the design history file). These pathogens do not exhibit sufficient sequence homology against the xTAG GPP primer sequences, and therefore would not be expected to cross-react with the exception of *Entamoeba coli and Taenia saginata*.

Table 17: *In silico* evaluation of pathogens for potential cross-reactivity

Pathogen
Ascaris lumbricoides (roundworm)
Chilomastix mesnili
Cryptosporidium canis
Cryptosporidium felis
Cyclospora cayetanensis
DF-3 – Dysgonomonas capnocytophagoides
Dientamoeba fragilis
Diphyllobothrium species
Endolimax nana
Entamoeba coli
Entamoeba hartmanni
Entamoeba polecki
Enterobius vermicularis (pinworm)
Enteromonas hominis
Hymenolepis nana (the dwarf tapeworm)
Idamoeba buetschlii
Isospora belli
Strongyloides stercoralis
Taenia sp.
Trichuris trichiura

From the in silico analysis, Entamoeba coli may cross-react with xTAG GPP primers based on the strong forward primer alignment of E_histolytica-FR_RVM77 (16 bp contig. on the 3' end) and reverse primer E_coli_stx1-Rev_Biosg_2 (10 bp contig. on the 3' end), as well as an amplimer size (138 bp) which is well within the design of the kit. To further elucidate, a thermal melting temperature (Tm) analysis was performed using the DINAMelt (Di-Nucleic Acid hybridization and melting prediction) program available at http://mfold.rna.albany.edu/?q=DINAMelt. Sequences of *Entamoeba coli* that aligned to the xTAG primers were analyzed to see if they would form a

stable interaction with the xTAG primers which could possibly result in cross reactivity with the xTAG GPP kit. Mismatches would negatively impact the Tm of the primers and *Entamoeba coli*. At the xTAG GPP reaction temperature of 58°C, the *Entamoeba coli* sequences would bind to the *E. histolytica* forward primer with approximately 64.4% of the *Entamoeba coli* sequences bound to the primer sequence, compared to binding of the forward primer to its target sequence without any mismatches (98.3%). However, binding of the reverse E. coli stx1 primer to *Entamoeba coli* would be reduced to 0.1% compared to this primer binding to its target sequence without any mismatches (81.8%). Therefore, *Entamoeba coli* is not likely to cross-react with the analytes in the xTAG GPP assay.

Fresh vs. Frozen

As in the original study results presented in k121894, results from the Fresh versus Frozen study using samples for the additional analytes are presented here. This evaluation generated data to demonstrate that there is no significant difference in the performance of xTAG GPP between specimens tested from the "fresh" state (i.e. unfrozen) and specimens that were tested after being stored frozen at -70°C to -80°C. Each of the three additional analytes, Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae* were assessed in a set of simulated specimens prepared in negative clinical matrix at a concentration close to the assay cut-off MFI (Low Positive), 5-10x the assay cut-off MFI (Moderate Positive) and, where possible, more than 10x the assay cut-off MFI (High Positive), where MFI is median fluorescent intensity value. Stability of un-extracted specimens, as well as pre-treated specimens, and finally, pre-treated and extracted nucleic acids were evaluated.

One Month Stability Results

Positive agreement between fresh and frozen un-extracted specimens was ≥ 95% with a lower bound of the 95% (two-sided) confidence interval exceeding 85% for Adenovirus 40/41 and *Vibrio cholerae*.

Positive agreement between fresh and frozen pre-treated specimens was ≥ 95% with a lower bound of the 95% (two-sided) confidence interval exceeding 85% for Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae*.

Positive agreement between fresh and frozen extracted specimens was ≥ 95% with a lower bound of the 95% (two-sided) confidence interval exceeding 85% for Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae.*

Adenovirus 40/41 and *Vibrio cholerae* met the 1-month stability acceptance criteria, and the MFIs generated on HP, MP and LP replicates of frozen un-extracted, extracted and extracted specimens were generally close to those generated at baseline. However, the un-extracted specimen stability of *Entamoeba histolytica* did not meet the acceptance criteria.

Three Month Stability Results

Positive agreement between fresh and frozen un-extracted specimens was ≥ 95% with a lower bound of the 95% (two-sided) confidence interval exceeding 85% for Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae*.

Positive agreement between fresh and frozen extracted specimens was ≥ 95% with a lower bound of the 95% (two-sided) confidence interval exceeding 85% for Adenovirus 40/41 and *Vibrio cholerae*.

The 3-month stability results for *Entamoeba histolytica* are of particular interest as they do not reflect the 1-month stability results. That is study criteria were met for the un-extracted specimen at 3-month stability time point but not at the 1-month time point. The 3-month stability data supports the stability of un-extracted *Entamoeba histolytica* frozen at -70°C to -80°C for 1 month. Study criteria for *Entamoeba histolytica* nucleic acid stability were met at the 1-month time point but not at the 3-month time point. Overall, the data supports the stability of un-extracted and extracted *Entamoeba histolytica* specimens frozen at -70°C to -80°C for 1 month.

Supplemental Stability Results - *Entamoeba histolytica* (un-extracted)
Additional data to support the stability of un-extracted *Entamoeba histolytica* specimens was also generated by analyzing LP and MP results obtained at site 1 (LMD) during the multi-site reproducibility study as well as testing LP and MP remnants at a later date. These results also suggest that un-extracted *Entamoeba histolytica* specimens are stable for at least 1-month when stored frozen at -70°C to -80°C.

Results are summarized for the un-extracted, pre-treated and extracted sample stability for the additional analytes in the following table.

Table 18: Summary of Stability Results Additional Analytes xTAG GPP (also see k121894)

Analyte Target	**Un-extracted 1 month**	**Un-extracted 3 months**	**Pre-Treated 1 month**	**Extracted 1 month**	**Extracted 3 months**
Adenovirus 40/41	√	√	√	√	√
Entamoeba histolytica	√^	√	√	√	X
Vibrio cholerae	√	√	√	√	√

^Based on supplemental testing results, possible titer or extraction issue with sample rather than stability failure

The results generated support the inclusion of frozen clinical specimens positive for all three targets, Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae,* in the multi-site clinical evaluation of the xTAG GPP. Results generated also indicate that pre-treated material and nucleic acid extracts of all three targets evaluated are stable for at least 1 month post freezing.

Precision / Reproducibility

Site-to-site reproducibility was assessed for each of the additional targets and for mixed analyte samples (representing co-infected samples). Original study results for the other analytes were presented in submission k121894. Replicates of simulated samples were tested across 3 sites by 2 operators at each site. One exception was made for testing of the *Vibrio cholerae* samples at Site 3, where due to operator illness the runs for the second operator were performed by two individuals. All sample replicates tested were prepared through serial dilutions of stock material (pre-treated negative stool spiked with a pathogen or positive stool) containing a microbial target from the intended use. Each sample replicate assayed in the study contained either a single microbial target or 2 microbial targets detected by xTAG GPP in addition to the internal control (bacteriophage MS2). For single analyte samples, dilutions tested fell into 1 of the following 3 categories:

1. High Negative (HN): microbial target concentrations which generate MFI values not lower than 20-30% below the cut-off MFI for the indicated analyte
2. Low Positive (LP): microbial target concentrations which generated MFI values that were 1-5X the cut-off MFI for the indicated analyte
3. Moderate Positive (MP): microbial target concentrations which generated MFI values 7-10X the cut-off MFI for the indicated analyte

For those samples prepared to simulate co-infections, one microbial target was present at the LP level defined above and the other at a High Positive (HP) level. HP levels were defined as follows:

High Positive (HP) viral cultures were prepared to a concentration of 10^5 PFU/mL (10^5 $TCID_{50}$/mL) or higher; High Positive (HP) bacterial cultures were prepared to a concentration of 10^6 CFU/mL or higher.

Each sample replicate underwent a single pre-treatment and extraction step. All samples were extracted using the NucliSens easyMAG extraction method. Extracted material was kept frozen at -70°C until testing. A total of 90 replicates were tested for each single analyte and dual analyte sample (3 replicates per run x 5 runs per operator x 2 operators per site x 3 sites = 90 replicates). Reproducibility was assessed both in terms of calls and MFI values.

Single Analyte Results

For single analyte samples prepared at the MP level, depending on the microbial target, 86/90 (95.6%) to 90/90 (100%) replicates generated a positive result (after allowable re-runs). For LP dilutions, depending on the microbial target, the correct positive call was made in 82/90 (91.1%) to 90/90 (100%) replicates tested. For HN dilutions, depending on the target, the correct negative call was generated in as few as 62/90 (68.9%) replicates to as many as 90/90 (100%). Greater variability in the HN dilution, compared to the LP and MP dilution, was expected based on the fact that a target is present in these samples at levels sufficient to generate MFI values 20-30% below the cut-off MFI, and based on the stochastic nature of end-point PCR in the presence of low levels of targeted analytes. Accordingly, percent variability, measured as the coefficient of variation (CV) for MFI values were lowest at the MP dilution and highest at the HN dilution. Results for single analyte samples are presented in Table 19.

Dual Analyte Results

For dual analyte samples tested for the additional targets (Table 20), all targets generated a positive call when present as a HP dilution. When present at the LP concentration, 2 of the 4 target combinations tested generated a positive call in 90/90 (100%) replicates tested. The 4 combinations were:
Rotavirus (HP) / Adenovirus (LP)
Adenovirus (HP) / Rotavirus (LP)
C. difficile (HP) / Adenovirus (LP)
Adenovirus (HP)/ *C. difficile* (LP)

C. difficile has two probes resulting in a single call for this target, (if either is positive, the target is positive). The following was observed for the remaining target present at LP concentration in the sample containing a second target at HP concentration:

- 4/90 replicates of the *C. difficile* (HP) /Adenovirus (LP) sample generated a negative call for Adenovirus
- 2/90 replicates of the Rotavirus (HP) / Adenovirus (LP) sample generated a negative call for adenovirus

It should be noted that although the *C. difficile* LP sample was 89/90 for probe 1, probe 2 made all the calls for the LP sample. Overall, adequate site-to-site reproducibility has been established for all targets that xTAG GPP has been designed to detect (also see results in k121894).

Table 19: Summary of Overall Total Raw Median MFI values for the Three Targets in xTAG GPP after Reruns

	Panel Member ID	Adenovirus 40/41 Low Positive	Adenovirus 40/41 Medium Positive	Adenovirus 40/41 High Negative	*Entamoeba histolytica* Low Positive	*Entamoeba histolytica* Medium Positive	*Entamoeba histolytica* High Negative	*Vibrio cholerae* Low Positive	*Vibrio cholerae* Medium Positive	*Vibrio cholerae* High Negative
	Concentration	**$1.45x10^{1}$ $TCID_{50}$/mL**	**$5.8x10^{1}$ $TCID_{50}$/mL**	**1.81 $TCID_{50}$/mL**	**$1.44x10^{1}$ Cells/mL**	**$5.76x10^{1}$ Cells/mL**	**$2.25x10^{-1}$ Cells/mL**	**$9.37x10^{6}$ CFU/mL**	**$3.75x10^{7}$ CFU/mL**	**$5.86x10^{5}$ CFU/mL**
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	20/30 66.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25^{th} Percentile MFI	732.5	1594.0	118.0	596.0	1366.0	43.0	616.5	1219.5	52.0
	Median MFI Value	797.0	1642.0	130.5	677.3	1475.5	45.5	691.3	1277.3	57.0
	75^{th} Percentile MFI	880.0	1692.0	160.0	783.5	1621.0	53.0	737.5	1364.0	69.0
	% CV	12.08	5.34	N/A	23.66	14.90	N/A	17.81	8.43	N/A
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	13/30 43.3%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	20/30 66.7%
	25^{th} Percentile MFI	740.0	1602.0	131.0	291.0	988.0	41.0	958.0	1579.0	66.0
	Median MFI Value	872.3	1748.8	170.3	423.3	1253.3	46.0	1256.5	1765.3	117.0
	75^{th} Percentile MFI	1046.0	1806.5	272.0	600.0	1573.5	58.0	1490.0	2001.5	172.0
	% CV	27.53	11.01	N/A	40.13	25.18	N/A	34.56	22.27	N/A
Site 3	Agreement with Expected Result	29/30 96.7%	29/30 96.7%	29/30 96.7%	22/30 73.3%	26/30 86.7%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25^{th} Percentile MFI	227.0	481.0	60.0	249.0	603.0	42.0	303.5	843.0	43.0
	Median MFI Value	287.0	648.5	69.0	352.8	778.5	43.5	373.5	1110.8	47.0
	75^{th} Percentile MFI	338.0	770.0	85.0	446.0	979.0	52.0	559.0	1210.0	58.0
	% CV	24.72	36.80	N/A	42.16	41.48	N/A	48.95	24.82	N/A
	Total Agreement with Expected Result	89/90 98.9%	89/90 98.9%	62/90 68.9%	82/90 91.1%	86/90 95.6%	90/90 100%	90/90 100%	90/90 100%	80/90 88.9%

	Panel Member ID	Adenovirus 40/41 Low Positive	Adenovirus 40/41 Medium Positive	Adenovirus 40/41 High Negative	*Entamoeba histolytica* Low Positive	*Entamoeba histolytica* Medium Positive	*Entamoeba histolytica* High Negative	*Vibrio cholerae* Low Positive	*Vibrio cholerae* Medium Positive	*Vibrio cholerae* High Negative
	Concentration	**$1.45x10^{1}$ $TCID_{50}$/mL**	**$5.8x10^{1}$ $TCID_{50}$/mL**	**1.81 $TCID_{50}$/mL**	**$1.44x10^{1}$ Cells/mL**	**$5.76x10^{1}$ Cells/mL**	**$2.25x10^{-1}$ Cells/mL**	**$9.37x10^{6}$ CFU/mL**	**$3.75x10^{7}$ CFU/mL**	**$5.86x10^{5}$ CFU/mL**
	95% CI	94.0%- 99.8%	94.0%- 99.8%	58.7%- 77.5%	83.4%- 95.4%	89.1%- 98.3%	95.9%-100.0%	95.9%-100.0%	95.9%-100.0%	80.7%- 93.9%
	Overall 25th Percentile MFI	338.0	770.0	80.5	319.0	937.0	42.0	435.0	1148.0	49.0
	Overall Median MFI Value	732.8	1596.0	127.8	459.5	1185.5	44.5	684.3	1297.8	58.5
	Overall 75th Percentile MFI	874.0	1722.0	167.5	667.0	1487.5	55.0	993.0	1610.5	89.0
	Overall % CV	48.08	38.52	N/A	44.47	35.79	N/A	52.32	30.36	N/A

Table 20: Summary of Overall Total Raw Median MFI values for Mixed Analytes in xTAG GPP after Reruns

	Panel Member ID	Rotavirus A Low Positive/ Adenovirus 40/41 High Positive		Rotavirus A High Positive/ Adenovirus 40/41 Low Positive		Adenovirus 40/41 Low Positive/ C. difficile High Positive			Adenovirus 40/41 High Positive/ C. difficile Low Positive		
		Rotavirus A Low Positive	Adenovirus 40/41 High Positive	Rotavirus A High Positive	Adenovirus 40/41 Low Positive	Adenovirus 40/41 Low Positive	C. difficile High Positive		Adenovirus 40/41 High Positive	C. difficile Low Positive	
							Probe 1	Probe 2		Probe 1	Probe 2
	Concentration	Indeterminate *	$9.28x10^2$ $TCID_{50}$/mL	Indeterminate *	$2.17x10^1$ $TCID_{50}$/mL	$2.17x10^1$ $TCID_{50}$/mL	$6.00x10^7$ CFU/mL	$6.00x10^7$ CFU/mL	$9.28x10^2$ $TCID_{50}$/mL	$7.50x10^6$ CFU/mL	$7.50x10^6$ CFU/mL
Site 1	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25th Percentile MFI	480.0	2198.0	1467.0	557.5	654.0	2216.5	3042.0	2529.0	577.0	1415.0
	Median MFI Value	1050.8	2313.0	1777.3	612.3	698.0	2540.0	3255.8	2618.0	730.0	1765.5
	75th Percentile MFI	1544.0	2407.5	1973.0	659.0	842.0	2756.5	3383.5	2785.0	875.0	1945.0
	% CV	71.36	5.85	28.29	15.37	21.80	17.84	7.66	6.38	40.08	23.43
Site 2	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%	30/30 100%
	25th Percentile MFI	443.5	2262.0	1626.0	413.5	400.0	2207.0	3043.0	2428.0	525.0	1636.0
	Median MFI Value	779.3	2501.0	1820.0	530.0	582.8	2547.5	3225.0	2540.3	599.0	1784.8
	75th Percentile MFI	1614.5	2709.0	2056.0	663.5	738.0	3029.0	3375.5	2827.0	1014.0	2012.5
	% CV	87.56	12.46	25.33	28.58	35.82	24.55	12.70	9.62	49.15	17.54
Site 3	Agreement with Expected Result	30/30 100%	30/30 100%	30/30 100%	28/30 93.3%	26/30 86.7%	30/30 100%	30/30 100%	30/30 100%	29/30 96.7%	30/30 100%
	25th Percentile MFI	440.0	1517.5	1130.0	198.0	218.0	972.0	2347.0	1570.5	281.5	1143.0
	Median MFI Value	719.5	1626.5	1299.5	239.8	259.5	1548.5	2516.0	1724.0	449.3	1278.5
	75th Percentile MFI	1153.0	1770.0	1577.5	280.0	280.0	1744.0	2632.0	1862.5	563.0	1427.0
	% CV	56.30	13.34	36.58	27.00	28.44	36.44	17.21	15.06	54.03	23.07
	Total Agreement with Expected Result	90/90 100%	90/90 100%	90/90 100%	88/90 97.8%	86/90 95.6%	90/90 100%	90/90 100%	90/90 100%	89/90 98.9%	90/90 100%
	95% CI	95.9%- 100.0%	95.9%- 100.0%	95.9%- 100.0%	92.3%- 99.4%	89.1%- 98.3%	95.9%- 100.0%	95.9%- 100.0%	95.9%- 100.0%	94.0%- 99.8%	95.9%- 100.0%
	Overall 25th Percentile MFI	443.5	1770.0	1291.0	280.0	280.0	1599.0	2580.0	1862.5	454.0	1311.0
	Overall Median MFI Value	762.5	2239.5	1662.5	470.5	512.0	2216.8	3042.5	2485.3	588.5	1639.3
	Overall 75th Percentile MFI	1207.0	2412.5	1943.5	624.0	710.5	2686.0	3305.0	2715.0	859.5	1890.0
	Overall % CV	76.97	20.60	31.77	41.43	49.01	34.27	18.18	21.27	50.99	25.77

*Real-time PCR failed to return a meaningful result. The amount of Rotavirus added to this sample is the same as the amount used in equivalent Rotavirus dilutions used in the Repeatability study.

Stool in Cary-Blair Media Limit of Detection Study Results

The purpose of this analytical study was to evaluate the equivalency in the limit of detection (LoD) between the two sample types: raw stool (sample type from k121894) and stool in Cary-Blair transport medium (additional sample type commonly collected) in a representative sub-set of the xTAG GPP targets. One analyte from each of three pathogen classes (bacterial, parasitic, and viral) was examined in the form of simulated stool samples and simulated stool samples in Cary-Blair media. The simulated samples were prepared as a dilution series using high titer stocks. The three representative analytes tested in this study were: *Clostridium difficile*, *Giardia lamblia* and Norovirus GII. Results of testing presented in Table 21 demonstrate that raw stool samples and stool samples in Cary-Blair media have equivalent limits of detection.

Table 21: Summary of the Limit of Detection (LoD) for GPP analytes in stool and stool in Cary-Blair media

Analyte	Strain ID	Raw Stool		Stool in Cary-Blair		LoD Difference between Stool and Stool in Cary-Blair
		Titer at limit of detection	Average MFI Value (n=20)	Titer at limit of detection	Average MFI Value (n=20)	
C. difficile Toxin A/B	*Clostridium difficile*, BAA-1805 (toxinotype III A+B+)	$4.69x10^5$ CFU/mL	Probe 1 = 363 Probe 2 = 784	$4.69x10^5$ CFU/mL	Probe 1 = 454 Probe 2 = 1097	None
Giardia	*Giardia lamblia,* PRA-243	$2.20x10^2$ cells/mL	658	$2.20x10^2$ cells/mL	633	None
Norovirus GI/GII	Norovirus GII, Clinical sample, source Toronto	$4.75x10^2$ copies/mL (Ct = 32.23)	1586	$4.75x10^2$ copies/mL (Ct = 32.23)	2781	None

Summary of negative control failures and sample re-run rates for analytical performance studies

Including all analytes in the xTAG GPP test intended use, there were a total of 284 xTAG GPP runs performed over the course of analytical performance studies. Each xTAG run has at least one no template negative control depending on batch size. Of the 284 runs, 15 (5.28%) had one or more negative control (NC) failures. These are summarized in the table below.

Table 22: Summary of Negative Control Failures for Analytical Performance Studies

Study	Total # of runs (including allowable re-runs)	Total # of runs with at least one NC failure	% total runs with at least one NC failure	Total No. of NCs included in runs and allowable re-runs	Total No. of NC failures	% total NC s included which failed in xTAG runs / allowable re-runs
Multi-site reproducibility	96	8	8.33%	249	10	4.02%
Matrix equivalence	3	0	0	9	0	0
Limit of detection	36	2	5.56%	119	2	1.68%
Carry-over contamination	9	0	0	0	0	0
Analytical specificity and interference	25	1	4.00%	101	1	0.99%
Analytical reactivity	34	1	2.94%	191	3	1.57%
Evaluation of fresh vs. frozen stool	81	3	3.70%	249	3	1.20%
Overall	**284**	**15**	**5.28%**	**918**	**19**	**2.07%**

Included in the 284 xTAG runs summarized above were 15455 specimens. Of these, 99.62% (15396/15455) yielded valid results on the first attempt. The remaining 59 specimens generated valid results following allowable re-runs. Sample re-run rates are summarized in the table below.

Table 23: Summary of Sample Re-Run Rates for Analytical Performance Studies

Studies	Total # of specimens tested	Total # of invalid results prior to re-run	% invalid results prior to re-run	Invalid results after re-run	% invalid results after re-run
Multi-site reproducibility	5065	25	0.49%	0	0.00%
Matrix equivalence	180	0	0.00%	0	0.00%
Limit of detection	972	2	0.21%	0	0.00%
Carry-over contamination	864	0	0.00%	0	0.00%
Analytical specificity and interference	1472	0	0.00%	0	0.00%
Analytical reactivity	2156	3	0.14%	0	0.00%
Evaluation of fresh vs. frozen stool	4746	29	0.61%	0	0.00%
Overall	**15455**	**59**	**0.38%**	**0**	**0.00%**

Clinical Performance:

Matrix Comparison Study

Unchanged, reference results in k121894

Detection in Asymptomatic Volunteers

In order to determine baseline levels for each analyte included in xTAG GPP for individuals who are not exhibiting signs and symptoms of infectious gastroenteritis, 200 clinical stool samples were collected from healthy, asymptomatic donors. Asymptomatic donors from various age groups were included in this study. Results presented below include the additional analytes in the xTAG GPP test. PCR inhibition, as determined by results for the internal control used with xTAG GPP (bacteriophage MS2), was observed in 30 of the 200 samples tested (15.0%). After re-running these specimens in accordance with the instructions for use, PCR inhibition was still observed in 7 samples (3.5%). The absence of a detectable internal control signal in these samples meant that negative results for the indicated microbial targets could not be reported. Therefore, the final data analysis was conducted on 193 of the 200 samples collected for this study.

Table 24: Percent negative results (including Adenovirus 40/41, *E. histolytica* and *V. cholerae*) and the analytes previously presented in the decision summary for k121894

Target	Percent Negative Results by xTAG® GPP for all samples	Percent Negative Results by xTAG® GPP for samples negative by sequencing
Adenovirus 40/41	100.0% (193/193)	100.0% (193/193)
Campylobacter	100.0% (193/193)	100.0% (193/193)
C. difficile toxin A/B	97.9% (189/193)[1]	99.0% (189/191)
Cryptosporidium	100.0% (193/193)	100.0% (193/193)
E. histolytica	99.5% (192/193)[2]	99.5% (192/193)
E. coli O157	100.0% (193/193)	100.0% (193/193)
ETEC LT/ST	100.0% (193/193)	100.0% (193/193)
Giardia	99.5% (192/193)[3]	99.5% (192/193)
Norovirus GI/GII	97.9% (189/193)*[4]	97.9% (189/193)
Rotavirus A	100.0% (193/193)	100.0% (193/193)
Salmonella	97.4% (188/193)[5]	97.4% (188/193)
STEC stx1/stx2	100.0% (193/193)	100.0% (193/193)
Shigella	99.5% (192/193)	99.5% (192/193)
V. cholerae	100.0% (193/193)	100.0% (193/193)

*NOTE: Sample 216 was positive by xTAG GPP for both Norovirus GII and *C. difficile*

1 Two (2) out of 4 xTAG GPP *C. difficile* positive samples were confirmed as positive by sequencing analysis.

2 The (1) xTAG GPP *E. histolytica* positive sample was not confirmed as positive by sequencing analysis.

3 None of the 2 xTAG GPP *Giardia* positive samples was confirmed as positive by sequencing analysis.

4 None of the 3 xTAG GPP Norovirus GI/GII positive samples was confirmed as positive by sequencing analysis.

5 None of the 5 xTAG GPP *Salmonella* positive samples was confirmed as positive by sequencing analysis.

As described in submission k121894, results of the study demonstrated ≥97% negative percent agreement across all analytes in the 193 samples tested. Samples (at the specimen level) that were positive by xTAG GPP but negative by sequencing were considered false positives (12/193). These samples had MFI values that were relatively close to the cut-offs. 2 samples at the specimen level that were called positive by xTAG GPP were also positive by sequencing for *C. difficile*. These two samples positive for *C. difficile* by both xTAG GPP and sequencing probably represent asymptomatic infections.

Clinical Cutoff

Not applicable

Detection in Symptomatic Patients (Prospective Clinical Study in Stool Specimens)

The clinical performance of xTAG GPP for each analyte probed by the assay was evaluated in clinical specimens (stools) prospectively collected between June 2011 and February 2012. A total of 1407 clinical specimens were collected from pediatric and adult patients and submitted for testing at six (6) independent laboratories. Four (4) of the laboratories were located in the United States (Arizona, Missouri, Tennessee and Texas) and two (2) were in Southern Ontario (Canada). Demographic details for this prospective data set were summarized in the original submission k121894. In this submission, results for the additional analytes (Adenovirus 40/41, *Entamoeba histolytica* and *Vibrio cholerae*) are provided for the prospective clinical study for samples collected in stool. Additionally, these same samples were also stored in Cary-Blair media, and results of testing these are also provided.

All prospective clinical specimens were analyzed by reference/comparator at central laboratories independent of xTAG GPP testing sites. Comparator methods were described in the original submission k121894 apart from the 3 listed below. For the additional analytes, the comparator methods are described in Table 25.

Table 25: Comparator Methods

xTAG® GPP analytes	Comparator Method
Adenovirus 40/41	Composite comparator consisting of Premier Adenoclone Type 40/41 EIA (Meridian Bioscience, K881894)^ directly on the stool specimen and Amplification + sequencing directly from clinical specimen using one NAAT†
Entamoeba histolytica	Microscopy followed by amplification + sequencing directly from clinical specimens using one NAAT† (positive specimens by microscopy only)
Vibrio cholerae	Bacterial culture

^ Meridian Bioscience acquired Cambridge Bioscience Corp. products

† NAAT, nucleic acid amplification test – see detailed description below

Clinical runs and re-runs using xTAG GPP were carried out on clinical specimens that had been extracted from the fresh or frozen state using the NucliSENS easyMAG method (bioMérieux, Inc.,

Durham, NC) according to the manufacturer's instructions. Total extracted nucleic acid material was stored at -70^{0}C prior to testing with xTAG GPP at each of the clinical sites. xTAG GPP positive results (expected values) for each individual target were summarized per age group in submission k121894, and are now summarized for the additional analytes in Table 26.

Table 26: Expected Values in stool specimens (As determined by xTAG GPP) – Summary by Age Groups for the xTAG GPP Prospective Clinical Evaluation (June 2011 – February 2012)

Target (Analyte)	Overall (n=1407)		0-1 year (n=6)		>1-5 years (n=20)		>5-21 years (n=76)		>21-65 years (n=879)		>65 years (n=426)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Adenovirus 40/41	21	1.5%	0	0.0%	2	10.0%	0	0.0%	12	1.4%	7	1.6%
Entamoeba histolytica	20	1.4%	0	0.0%	0	0.0%	1	1.3%	14	1.6%	5	1.2%
Vibrio cholerae	3	0.2%	0	0.0%	0	0.0%	0	0.0%	2	0.2%	1	0.2%

Accuracy determinations (diagnostic sensitivity and specificity, positive and negative agreement) were based on the fraction of comparator positive (or negative) results which were also positive (or negative) by xTAG GPP. Sensitivity (or positive agreement) was calculated by dividing the total number of "true positive" xTAG GPP results (TP) by the sum of the TP and "false negative" (FN) xTAG GPP results. Specificity (or negative agreement) was calculated by dividing the total number of "true negative" xTAG GPP results (TN) by the sum of the TN and "false positive" (FP) xTAG GPP results. An xTAG GPP result was considered to be a TP or TN result only in the event that it agreed with the comparator method result for the analyte in question. 95% confidence intervals were calculated using the Wilson score method.

Since the reagents in the xTAG Kit remain the same, data from the original clinical study (k121894) are still applicable. Tables 27-29 present the stool results for each of the additional analyte targets added to the intended use of xTAG GPP for the clinical prospective sample set (N=1407).

Table 27: 3X3 for Adenovirus 40/41 (stool)

xTAG GPP	Primary Comparator			
	Positive	Negative	Invalid	TOTAL
Positive	4	17	0	21
Negative	1[1]	1158	0	1159
Invalid	2	225	0	227
TOTAL	7	1400	0	1407
		95% CI		
Positive Agreement	80%	37.5% - 96.4%		
Negative Agreement	98.5%	97.7% - 99.1%		
Invalid Rate	16.1%			

[1] The one specimen that was positive for Adenovirus 40/41 by comparator but negative by xTAG GPP was positive by bi-directional sequencing only (i.e. FDA-cleared EIA negative).

Table 28: 3X3 for *Entamoeba histolytica* (stool)

xTAG GPP	**Primary Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	0	20	0	20
Negative	0	1154	0	1154
Invalid	0	233	0	233
TOTAL	0	1407	0	1407
		95% CI		
Sensitivity	N/A	N/A		
Specificity	98.3%	97.4% – 98.9%		
Invalid Rate	16.6%			

Table 29: 3X3 for *Vibrio cholerae* (stool)

xTAG GPP	**Primary Comparator**			
	Positive	Negative	Invalid	TOTAL
Positive	0	3	0	3
Negative	0	1171	0	1171
Invalid	0	233	0	233
TOTAL	0	1407	0	1407
		95% CI		
Sensitivity	N/A	N/A		
Specificity	99.7%	99.2% – 99.9%		
Invalid Rate	16.6%			

A summary of the prospective clinical performance data in human stool specimens (from k121894 and this submission) is presented for each of the analytes in Table 30 below.

Table 30: Summary of Prospective Performance Data (N=1407) testing human stool specimens including Adenovirus 40/41, E. histolytica and V. cholerae, with the results from k121894

Analyte	Sensitivity			Specificity			Number Invalid xTAG GPP Results due to PCR Inhibition
	TP / (TP+FN)	percent	95%CI	TN / (TN+FP)	percent	95%CI	
Campylobacter	3/3	100%	43.9% - 100%	1161/1183	98.1%	97.2% – 98.8%	221
Cryptosporidium	12/13	92.3%	66.7% – 98.6%	1132/1189	95.2%	93.8% – 96.3%	205
E. coli O157	2/2	100%	34.2% - 100%	1163/1174	99.1%	98.3% – 99.5%	231
Entamoeba histolytica	0/0	N/A	N/A	1154/1174	98.3%	97.4% – 98.9%	233
Giardia	4/4	100%	51.0% - 100%	1138/1175	96.9%	95.7% – 97.7%	228
Salmonella	10/10	100%	72.2% - 100%	1145/1164	98.4%	97.5% – 99.0%	233
Shigella	2/2	100%	34.2% - 100%	1160/1178	98.5%	97.6% – 99.0%	227
Vibrio cholerae	0/0	N/A	N/A	1171/1174	99.7%	99.2% – 99.9%	233
Analyte	**Positive Agreement**			**Negative Agreement**			**Number Invalid xTAG GPP Results due to PCR Inhibition**
	TP / (TP+FN)	**percent**	**95%CI**	**TN / (TN+FP)**	**percent**	**95%CI**	
Adenovirus 40/41	4/5	80%	37.5% - 96.4%	1158/1175	98.5%	97.7% – 99.1%	227
C. difficile Toxin A/B[1]	107/114	93.9%	87.9% – 97.0%	921/1035	89.0%	86.9% - 90.8%	163
ETEC	2/8	25.0%	7.1% - 59.1%	1161/1166	99.6%	99.0% - 99.8%	233
Norovirus GI/GII	74/78	94.9%	87.5% – 98.0%	1022/1121	91.2%	89.4% - 92.7%	208
Rotavirus A	2/2	100%	34.2% - 100%	1167/1170	99.7%	99.2% - 99.9%	235
STEC	1/1	100%	20.7% - 100%	1160/1175	98.7%	97.9% - 99.2%	231

[1]A total of 95 specimens generated a "Nonspecific reaction, not characteristic of *Clostridium difficile* toxin. A titration test was performed on all 95 specimens and it was determined that in each case, the cytotoxicity reaction was not typical of *C. difficile* toxin.

When all of the analyte data is combined, xTAG GPP detected a total of 97 mixed infections in the prospective clinical evaluation. This represents 19.4% of the total number of xTAG GPP positive specimens (97/501). 58 (58/97; 59.8%) were double infections, 26 (26/97; 26.8%) were triple infections, 7 (7/97; 7.2%) were quadruple infections, 2 (2/97; 2.1%) was sextuple infection and 4 were infected by 7 or more pathogens (4/97; 4.1%). The single most common co-infections (23/97; 23.7%) was Norovirus GI/GII with *C. difficile* Toxin A/B. Out of the 97 co-infections, 92 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections.

Detection in Symptomatic Patients (Prospective Clinical Study in Stool in Cary-Blair Media)

Original comparator method test results for all samples in the prospective study (see k121894) were utilized for comparison to stool samples in Cary-Blair media for which adequate sample was available. The purpose of the study was to establish diagnostic accuracy of xTAG GPP in stool specimens in Cary-Blair medium. Clinical performance (sensitivity/positive percentage agreement and specificity/negative percentage agreement) of xTAG GPP on stool in Cary-Blair medium is summarized for each individual target in Table 31 below. For comparison purposes, clinical performance results generated from the unpreserved stool as part of the original clinical study are also presented.

Table 31: Summary of xTAG GPP Clinical Performance

Target	Sensitivity						Specificity					
	Unpreserved Stool			Stool in Cary-Blair Media			Unpreserved Stool			Stool in Cary-Blair Media		
	TP/(TP+FN)	%	95% CI	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI	TN/(TN+FP)	%	95% CI
Campylobacter	3/3	100.0%	43.9% - 100%	3/3	100.0%	43.9% - 100%	1161/1183	98.1%	97.2% – 98.8%	1284/1293	99.3%	98.7% - 99.6%
Cryptosporidium	12/13	92.3%	66.7% - 98.6%	12/13	92.3%	66.7% - 98.6%	1132/1189	95.2%	93.8% – 96.3%	1259/1288	97.7%	96.8% - 98.4%
Entamoeba histolytica	n/a			n/a			1154/1174	98.3%	97.4% – 98.9%	1276/1298	98.3%	97.5% - 98.9%
E. coli O157	2/2	100.0%	34.2% - 100%	2/2	100.0%	34.2% - 100%	1163/1174	99.1%	98.3% – 99.5%	1287/1294	99.5%	98.9% - 99.7%
Giardia	4/4	100.0%	51.0% - 100%	4/4	100.0%	51.0% - 100%	1138/1175	96.9%	95.7% – 97.7%	1275/1298	98.2%	97.4% - 98.8%
Salmonella	10/10	100.0%	72.2% - 100%	10/10	100.0%	72.2% - 100%	1145/1164	98.4%	97.5% – 99.0%	1255/1289	97.4%	96.3% - 98.1%
Shigella	2/2	100.0%	34.2% - 100%	2/2	100.0%	34.2% - 100%	1160/1178	98.5%	97.6% – 99.0%	1291/1296	99.6%	99.1% - 99.8%
Vibrio cholerae	n/a			n/a			1160/1178	98.5%	97.6% – 99.0%	1296/1296	100%	99.7% -100%

Target	Positive Agreement						Negative Agreement					
	Unpreserved Stool			Stool in Cary-Blair Media			Unpreserved Stool			Stool in Cary-Blair Media		
	TP/(TP+FN)	%	95% CI	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI	TN/(TN+FP)	%	95% CI
Adenovirus 40/41[1]	4/5	80.0%	37.5% - 96.4%	2/6	33.3%	9.7% - 70.0%	1158/1175	98.5%	97.7% – 98.1%	1285/1290	99.6%	99.1% - 99.8%
Clostridium difficile toxin A/B	107/114	93.9%	87.9% - 97.0%	98/108	90.7%	83.8% - 98.9%	921/1035	89.0%	86.9% - 90.8%	1027/1118	91.9%	90.1% - 93.3%
ETEC LT/ST[2]	2/8	25.0%	7.1% - 59.1%	2/9	22.2%	6.3% - 54.7%	1161/1166	99.6%	99.0% - 99.8%	1283/1287	99.7%	99.2% - 99.9%
Norovirus GI/GII	74/78	94.9%	87.5% - 98.0%	70/73	95.9%	88.6% - 98.6%	1022/1121	91.2%	89.4% - 92.7%	1153/1224	94.2%	92.8% - 95.4%
Rotavirus A	2/2	100.0%	34.2% - 100%	2/2	100.0%	34.2% - 100%	1167/1170	99.7%	99.2% - 99.9%	1294/1294	100%	99.7% - 100%
STEC	1/1	100.0%	20.7% - 100%	1/1	100.0%	20.7% - 100%	1160/1175	98.7%	97.9% - 99.2%	1290/1296	99.5%	99.0% - 99.8%

[1]In the case of Adenovirus 40/41, one of the clinical specimens that was concordant positive in the original GPP runs performed on raw stool yielded a negative result when tested in Cary-Blair. MFI generated in the original stool run were close to the assay cut off (176) suggesting a low titer specimen. Two other specimens that were inhibited in the original stool runs performed on raw stool yielded a negative result in the Cary-Blair runs. Lastly, one specimen that was positive for Adenovirus 40/41 by composite comparator was unavailable for re-testing in the Cary-Blair study. For these reasons, positive agreement of xTAG GPP for Adenovirus 40/41 dropped from 80% (4/5) in the raw stool study to 33.3% (2/6) in the Cary-Blair evaluation.

[2]ETEC comparator results were calculated against a composite consisting of four well characterized nucleic acid amplification tests (NAATs) followed by bi-directional sequencing. All specimens that were false negative by xTAG GPP for ETEC were positive by only one out of four comparator NAATs. Repeat sequencing of these specimens were negative by all four NAAT, except for one sample which was positive by one NAAT.

Clinical sensitivity or positive agreement acceptance criterion of 90% with a lower bound 95% confidence interval of at least 80% was achieved for Norovirus GI/GII and *Clostridium difficile* toxin A/B on stool in Cary-Blair media. The results were equivalent to those obtained for unpreserved stool specimens. Similar to the unpreserved stool, the lower bound 95% confidence interval for sensitivity was not met for all other targets probed by xTAG GPP on stool in Cary-Blair media. This can be explained by the low positivity rate in the prospective sample set.

Although a smaller sample set was used for the pre-selected arm of the study, positive agreement between comparator and xTAG GPP results was 100% for all pre-selected targets tested. Clinical specificity or negative percentage agreement acceptance criterion of 90% with a lower bound 95% confidence interval of at least 90% were achieved for all targets probed by xTAG GPP.

Other Supportive Clinical Data

Pre-selected stool specimens Retrospective Study

A total of 207 archived stool specimens that were positive by reference/comparator for pathogens that were of low prevalence in the prospective clinical study were collected at multiple sites in North America, Africa and Europe. Luminex was unable to source any stool specimens that tested positive for *Vibrio cholerae* by reference method for the pre-selected arm of the study. As previously noted, the range of analyte concentrations in these pre-selected specimens represented the clinically relevant range of concentrations observed in patients with gastrointestinal infection. All pre-selected positive specimens were tested with xTAG GPP at 4 sites (3 of which were external to LMD), along with negative clinical specimens in a randomized, blinded fashion. The "negative" designation for these specimens was based on the routine algorithms used at the banking site (e.g. bacterial culture, EIA, microscopy, in-house real time PCR). These algorithms did not test for all pathogen targets probed by xTAG GPP. Table 32 summarizes the positive agreement between reference/comparator and xTAG GPP for all pre-selected targets evaluated.

Table 32: Positive Percent Agreement of xTAG GPP in the Pre-selected Stool Data Set

Target	Positive Agreement		95%CI for Positive Agreement	Number Invalid xTAG GPP Results
	TP / (TP+FN)	Percent		
Adenovirus 40/41	3/3	100%	43.8% - 100%	0
Campylobacter	40/41	97.6%	87.4% - 99.6%	0
Cryptosporidium	12/12	100%	75.7% - 100%	1
Entamoeba histolytica	1/1	100%	2.5% - 100%	0
E. coli O157[1]	14/14	100%	78.5% - 100%	0
ETEC	38/39	97.4%	86.8% - 99.5%	0
Giardia[2]	15/16	93.7%	71.7% - 98.9%	1
Rotavirus A	28/28	100%	87.9% - 100%	0

Salmonella	24/27	88.89%	71.9% - 96.1%	0
STEC[3]	18/18	100%	82.4% - 100%	0
Shigella	20/20	100%	83.9% - 100%	0

1- Eight (8)/8 *E. coli 0157* were also positive for STEC by xTAG GPP. Sample remnants of all 8 *E. coli* 0157 specimens were tested for the presence of stx1 and stx2 genes by bi-directional sequencing and the results added to those obtained for STEC.
2- One (1) false negative *Giardia* specimen was reported. This specimen was also negative for *Giardia* by in-house real-time PCR performed at the site.
3- Six (6)/10 STEC were also positive for *E. coli* 0157 by xTAG GPP. Sample remnants of all 10 STEC specimens were assessed by bi-directional sequencing for *E. coli* 0157 and the results added to those obtained for *E. coli* 0157.

Confirmatory testing by nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was also conducted on all available specimens tested in the pre-selected arm of the clinical study. More specifically, confirmatory testing was performed for those analytes that were positive by xTAG GPP but not pre-selected at the banking site in order to determine whether these additional positive calls represented True Positive (TP) or False Positive (FP) clinical results. To the extent possible, sequencing primers targeted genomic regions distinct from those of the kit primers. xTAG GPP generated 122 additional positive calls (after allowable re-runs) for analytes that were not pre-selected at the banking site. Results of confirmatory testing from the preselected study were presented in the submission summary k121894, and the additional analyte results only are presented here. Sequencing primer validation studies were also presented in the submission summary k121894 and are not repeated here.

Table 33: 3X3 Table for Additional Adenovirus 40/41 Confirmatory Testing Results – Pre-selected Stool Sample Set

xTAG GPP	**PCR/Bi-directional Sequencing**			
	Positive	Negative	Not Done	TOTAL
Additional Positive	5	2	0	7
Negative	N/A	N/A	403	403
Invalid	N/A	N/A	67	67
TOTAL	5	2	470	477
Confirmed Positive	71.4%			
Invalid Rate (N=480)	13.9%			

Table 34: 3X3 Table for Additional *Entamoeba histolytica* Confirmatory Testing Results – Pre-selected Stool Sample Set

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Additional Positive	1	6	1	8
Negative	N/A	N/A	404	404
Invalid	N/A	N/A	67	67
TOTAL	1	6	472	479*
Confirmed Positive	12.5%			
Invalid Rate (N=480)	13.9%			

* 1 specimen was pre-selected for *Entamoeba histolytica*.

Table 35: 3X3 Table for Additional *Vibrio Cholerae* Confirmatory Testing Results – Pre-selected Stool Sample Set

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Additional Positive	0	0	0	0
Negative	N/A	N/A	413	413
Invalid	N/A	N/A	67	67
TOTAL	0	0	480	480
Confirmed Positive	N/A			
Invalid Rate (N=480)	13.9%			

xTAG GPP detected a total of 73 mixed infections in the pre-selected arm of the clinical study. This represents 29.9% of the total number of xTAG GPP positive specimens (73/244). 59 (59/73: 80.8%) were double infections, 13 (13/73: 17.8%) were triple infections and 1 was quadruple infection (1/73; 1.4%). The single most common co-infections (excluding *E. coli* 0157 with STEC; N=12) was ETEC with Shigella (6/73; 8.2%). Out of the 73 co-infections, 26 contained one or more analytes that was not confirmed by bi-directional sequencing, i.e. discrepant co-infections. All mixed infection combinations detected by the reference/comparator methods were detected by xTAG GPP.

Pre-selected Stool in Cary-Blair Specimens Retrospective Study

Remnants of available pre-selected frozen stool specimens tested as part of the original clinical study were mixed proportionally with Cary-Blair medium and tested in a randomized, blinded fashion. Results are presented in the table below.

Table 36: Positive percent agreement of xTAG GPP in the pre-selected Cary-Blair

Target	Positive Agreement		95% Confidence Interval (CI)	Number of Invalid Results
	TP/(TP+FN)	Percentage		
Campylobacter	40/40	100.0%	91.3% - 100%	0
E. coli O157	2/2	100.0%	34.2% - 100%	0

Salmonella	26/26	100.0%	87.1% - 100%	0
Shigella	13/13	100.0%	77.2% - 100%	0

Supplemental Clinical Data (Simulated Stool Specimen Results)

Due to difficulties in sourcing a sufficient number of retrospective stool specimens positive by reference method for *Entamoeba histolytica* and *Vibrio cholerae,* the performance of the xTAG GPP assay for these targets was further evaluated on contrived samples made using individual stool matrix spiked with varying levels of pathogen representing both the clinically relevant concentrations and concentrations that challenge the Limit of Detection (LoD) of the xTAG GPP assay. The results of testing are provided below (Table 37) and met study acceptance criteria.

Table 37: Summary of the Results Obtained for the Analyte Positive Contrived Specimens

Target	Concentration	Agreement with Expected Result	Mean MFI Value	% CV	95% CI*
Entamoeba histolytica	$5.76x10^1$ cells/mL	25/25 (100%)	2419	76.7%	
	$1.23x10^2$ cells/mL	1/1 (100%)	2330	N/A	
	$3.96x10^2$ cells/mL	1/1 (100%)	2973	N/A	
	$1.23x10^3$ cells/mL	2/2 (100%)	2444	N/A	
	$1.23x10^4$ cells/mL	2/2 (100%)	2383	N/A	
	$1.65x10^4$ cells/mL	5/5 (100%)	3086	18.1%	
	$4.00x10^4$ cells/mL	4/4 (100%)	2367	24.5%	
	$1.20x10^5$ cells/mL	4/4 (100%)	2290	30.1%	
	$4.00x10^5$ cells/mL	3/3 (100%)	2813	27.2%	
	$4.00x10^6$ cells/mL	3/3 (100%)	2673	21.8%	
***Entamoeba histolytica* Overall Positive Percent Agreement**		**50/50 (100%)**			**92.9%-100%**
Negative Percent Agreement		**100/100 (100%)**			**96.1%-100%**
Vibrio cholerae	$4.86x10^6$ CFU/mL	25/25 (100%)	1619	26.4%	
	$1.00x10^7$ CFU/mL	4/5 (80%)	1448	55.9%	
	$3.00x10^7$ CFU/mL	5/5 (100%)	1821	19.6%	
	$1.00x10^8$ CFU/mL	5/5 (100%)	1405	27.2%	
	$3.00x10^8$ CFU/mL	5/5 (100%)	1563	27.7%	
	$6.00x10^8$ CFU/mL	5/5 (100%)	1429	25.8%	
***Vibrio cholerae* Overall Positive Percent Agreement**		**49/50 (98%)**			**89.5%-99.7%**
Negative Percent Agreement		**100/100 (100%)**			**96.1%-100%**

*Confidence intervals (CI) calculated using CI calculator available online at http://www.vassarstats.net/prop1.html

The 50 *Entamoeba histolytica* contrived stool specimens had 100% (50/50) concordance with the expected positive result. The signals for the *Entamoeba histolytica* positive calls ranged from 299 MFI – 5327 MFI and the internal control signal (MS2) was present for all specimens. The 50 *Vibrio cholerae* contrived stool specimens had 98% (49/50) concordance with the expected positive result. The one sample that did not call positive hand a signal of 73 MFI, which is near the positive call threshold of 150 MFI. The signal range for all *Vibrio cholerae* contrived stool specimens was 73 MFI – 2328 MFI; the signal range for the specimens which called positive for *Vibrio cholerae* was 899MFI – 2328MFI. All contrived negative stool specimens (N=50) produced the expected negative result for all analytes. The internal control (MS2) was present in all the negative contrived stool specimens and produced a signal range of 210 MFI – 1463 MFI.

Supplemental Clinical Data (Simulated Stool in Cary-Blair Specimen Results)

In order to assess whether Cary-Blair results generated for Adenovirus 40/41 in the prospective study were an accurate representation of the performance of xTAG GPP for this target, contrived specimens made from individual negative stool specimens in Cary-Bair were prepared at concentration spanning the analytical detection range of the assay and tested in a randomized fashion with negative specimens. Both Adenovirus 40 and 41 cultured isolates were tested and 50% of the samples were prepared at a concentration of 2x LoD. Results of this evaluation are presented in the table below (Table 38).

Table 38. Summary of the results for Adenovirus 40/41 stool in Cary-Blair contrived samples

Target	Source	Strain	Titer ($TCID_{50}$/mL)	Multiples of LoD (approximated based on real-time PCR assay)	Number of Contrived Samples	Agreement with Expected Positive Results	95% Confidence Interval (CI)
Adenovirus 40	ATCC	Type 40 (Dugan)	2.90×10^1	2X	13	100% (13/13)	
			2.32×10^2	16X	6	100% (6/6)	
			9.28×10^2	64X	6	100% (6/6)	
Adenovirus 40 Overall					25	100% (25/25)	86.7% -100%
Adenovirus 41	Zeptometrix	Type 41 (Tak)	1.54×10^1	2X	12	100% (12/12)	
			1.23×10^2	16X	7	100% (7/7)	
			4.92×10^2	64X	6	100% (6/6)	
Adenovirus 41 Overall					25	100% (25/25)	86.7% -100%
Adenovirus 40/41 Overall					**50**	**100% (50/50)**	**92.9% - 100%**

In addition, due to the limited number of *Entamoeba histolytica* and *Vibrio cholerae* clinical samples available for testing during the clinical study, an additional study of contrived specimens in Cary-Blair was performed. A total of 150 stool in Cary-Blair contrived specimens consisting of 50 negative specimens, 50 specimens positive for *Entamoeba histolytica* and 50 specimens positive for *Vibrio cholerae* were analyzed with the xTAG GPP assay. Contrived specimens in Cary-Blair were prepared in the same manner as contrived stool specimens (see above). Results of this evaluation are summarized in the table below.

Table 39. Summary of the results obtained for the analyte positive stool in Cary-Blair contrived specimens

Target	Concentration	Agreement with Expected Result	Mean MFI Value	% CV	95% CI*
Entamoeba histolytica	$5.76x10^1$ Cells/mL	22/24 (92%)	1581	58%	
	$4.61x10^2$ Cells/mL	5/5 (100%)	2652	28%	
	$9.22x10^2$ Cells/mL	5/5 (100%)	3386	5%	
	$1.84x10^3$ Cells/mL	5/5 (100%)	3257	17%	
	$1.00x10^4$ Cells/mL	5/5 (100%)	2958	31%	
	$3.00x10^4$ Cells/mL	5/5 (100%)	3063	38%	
***Entamoeba histolytica* Overall**		**47/49 (96%)**			**86.3% - 98.9%**
Vibrio cholerae	$4.68x10^6$ CFU/mL	25/25 (100%)	1360	30%	
	$1.00x10^7$ CFU/mL	5/5 (100%)	2245	2%	
	$3.00x10^7$ CFU/mL	5/5 (100%)	2107	23%	
	$1.00x10^8$ CFU/mL	9/9 (100%)	2238	22%	
	$3.00x10^8$ CFU/mL	6/6 (100%)	2079	26%	
***Vibrio cholerae* Overall**		**50/50 (100%)**			**92.9% - 100%**

*Confidence intervals (CI) calculated using CI calculator available online at http://www.vassarstats.net/prop1.html

Supplemental Clinical Data (Botswana Pediatric Stool Specimens)

The clinical performance of xTAG GPP for Adenovirus 40/41, Rotavirus, ETEC, *Cryptosporidium* and *Giardia* was also evaluated in a set of pediatric stool specimens (N=313) prospectively collected between February 2011 and January 2012 from symptomatic pediatric patients admitted to two referral hospitals in Botswana, Africa. All pediatric patients included in this evaluation presented with diarrhea and/or vomiting. All specimens were shipped frozen to a testing site located in Southern Ontario (Canada). As described and presented in k121894, comparator testing by nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was performed on samples positive for Adenovirus 40/41, Rotavirus, ETEC, *Cryptosporidium* and *Giardia* by xTAG GPP. In order to minimize bias, a random subset of the Botswana cohort that tested negative by xTAG GPP was assessed by the same nucleic acid amplification followed by bi-directional sequencing method for Rotavirus, ETEC, *Cryptosporidium and Giardia.* In addition, all available clinical specimens (N=311) were assessed for Adenovirus 40/41 using the same FDA-cleared EIA as that used in the prospective study (Premier Adenoclone Type 40/41 EIA, Meridian Bioscience, K881894). Results for Adenovirus 40/41 are presented below. Results for other analytes were previously presented in the submission summary for k121894.

Table 40: 3X3 Table for Adenovirus 40/41 - Botswana Stool Sample Set

xTAG GPP	Adenovirus Type 40/41 EIA			
	Positive	Negative	Invalid	TOTAL
Positive	35	0	0	35
Negative	17	255	0	272
Invalid	1	5	0	6
TOTAL	53	260	0	313
		95% CI		
Positive Agreement	67.3%	53.7% - 78.5%		
Negative Agreement	100%	98.5% - 100%		
Invalid Rate [2]	1.9%			

[1] All 17 specimens that were positive for Adenovirus 40/41 by comparator but negative by xTAG GPP were positive by bi-directional sequencing only (i.e. FDA-cleared EIA negative). All these 17 specimens were assessed by real-time PCR for Adenovirus (all sub-types) at the laboratory testing site. The mean Ct value for these 17 specimens was 33.1; indicating low viral titer in these specimens, which is less clinically relevant.

[2] All these 35 specimens were also assessed by real-time PCR for Adenovirus (all sub-types) at the laboratory testing site. In contrast to the 17 specimens in footnote 1 above, the mean Ct value for the 35 adenovirus samples positive by the PCR/Bi-directional sequencing assay and detected by xTAG GPP in this cohort was 21.38; indicating higher viral titer in these specimens, which is more clinically relevant.

[3] 222 of the comparator negative Adenovirus 40/41 specimens were assessed by FDA-cleared EIA only.

[4] Six out of a total of 313 samples tested by the xTAG GPP generated an "invalid" result for Adenovirus 40/41.

Nucleic acid amplification followed by bi-directional sequencing using analytically validated primers was also performed on all available clinical specimens that were positive by xTAG GPP for other analytes. The tables below summarize the confirmed xTAG GPP positive rate (i.e., confirmed xTAG GPP positives/all xTAG GPP positives) by PCR/bi-directional sequencing for *Entamoeba Histolytica* and *Vibrio cholerae*. Results for *Campylobacter*, *C. difficile* Toxin A/B, *E. coli* O157, Norovirus, *Salmonella*, *Shigella*, and STEC were previously presented in the submission summary for k121894.

Table 41: 3X3 Table for *Entamoeba Histolytica* - Botswana Stool Sample Set

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	0	0	0	0
Negative	NA	NA	307	307
Invalid	NA	NA	6	6
TOTAL	0	0	313	313
Confirmed Positive Rate	N/A			
Invalid Rate	1.9%			

Table 42: 3X3 Table for *Vibrio cholerae* - Botswana Stool Sample Set

xTAG GPP	PCR/Bi-directional Sequencing			
	Positive	Negative	Not Done	TOTAL
Positive	0	0	0	0
Negative	NA	NA	307	307
Invalid	NA	NA	6	6
TOTAL	0	0	313	313

Confirmed Positive Rate	N/A			
Invalid Rate	1.9%			

xTAG GPP detected a total of 115 mixed infections in the Botswana study. This represents 40.5% of the total number of xTAG GPP positive specimens (115/284). 8 8 (88/115; 76.5%) were double infections, 20 (20/115; 117.4%) were triple infections, 5 (5/115; 4.3%) were quadruple infections and 2 (2/115; 1.7%) were quintuple infections. The single most common co-infection was Rotavirus with *Campylobacter* (18/115; 15.6%). Out of the 115 co-infections, 22 contained one or more analytes that was not confirmed by bi-directional sequencing, i.e. discrepant co-infections.

A summary of the specimen failure rates are summarized for each clinical study in Table 43 below.

Table 43: Summary of Sample Failure Rates in Clinical Performance Studies

Clinical Studies	**Total # of specimens tested**	**Sample Failure due to PCR Inhibition**		**Sample Failure due to PCR Contamination**	
		# Re-runs	**% Re-runs**	**# Re-runs**	**% Re-runs**
Prospective Study	1407	236	16.8%	56	4.0%
Pre-selected Study	480	67	14.0%	30	6.2%
Botswana Study	313	5	1.6%	80	25.6%

Expected Values / Reference Range

In addition to the Expected Values information for additional analytes presented in Table 26 above (summary by age groups), Table 44 details the expected values by site. Expected values for other analytes were presented in the decision summary for k121894.

Table 44: Expected Values (As determined by xTAG GPP) – Summary by Site for the xTAG GPP Prospective Clinical Evaluation (Jun 2011 to Feb. 2012)

Target (Analyte)	Overall (n=1407)		Site 1 (n=434)		Site 2 (n=428)		Site 3 (n=155)		Site 4 (n=260)		Site 5 (n=88)		Site 6 (n=42)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Adenovirus 40/41	21	1.5%	9	2.1%	9	2.1%	0	0.0%	3	1.2%	0	0.0%	0	0.0%
Entamoeba histolytica	20	1.4%	6	1.4%	8	1.9%	2	1.3%	2	0.8%	2	2.3%	0	0.0%
Vibrio cholerae	3	0.2%	0	0.0%	3	0.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Instrument and System Information
Luminex MAGPIX with xPONENT Software

1. Modes of Operation: See Device Description above.

2. Software: Hazard Analysis included in original k121894 submission documentation

3. Specimen Identification:
Users must fill in Batch Information by providing a unique batch Name, Description and Creator. Users have to enter appropriate patient information, i.e. number of samples, and sample IDs.

4. Specimen Sampling and Handling:
DNA is extracted using the bioMérieux NucliSENS easyMAG system. Samples are manually prepared for amplification according to assay package insert and, once amplified, are transferred to a 96-well microtiter plate for analysis on the Luminex system.

5. Calibration:
The Luminex MAGPIX Calibration Kit is intended to calibrate the optics of the MAGPIX instrument. During calibration, the system adjusts LED current and calibration factors for CL1, CL2, and RP1 until those values match the imported target values, thus calibrating the classification map. This product is not intended to be used in place of the assay calibrators or assay controls that are required to verify the proper function of a given assay.

6. Quality Control:
The Luminex MAGPIX Performance Verification Kit is intended to verify the optical calibration of the MAGPIX instrument. This product is not intended to be used in place of the assay calibrators or assay controls that are required to verify the proper function of a given assay.